Title	EXPERIMENTAL STUDY OF THE INTERRELATION BETWEEN THE PINEAL BODY AND THE HYPOPHYSIS, WITH PARTICULAR REFERENCE TO THE SOMATOSEXUAL DEVELOPMENT
Author(s)	Kageyama, Naoki
Citation	日本外科宝函 (1955), 24(5): 470-487
Issue Date	1955-09-01
URL	http://hdl.handle.net/2433/206211
Right	
Type	Departmental Bulletin Paper
Textversion	publisher

EXPERIMENTAL STUDY ON THE INTERRELATION BETWEEN THE PINEAL BODY AND THE HYPOPHYSIS, WITH PARTICULAR REFERENCE TO THE SOMATOSEXUAL DEVELOPMENT

by

NAOKI KAGEYAMA

(from the First Surgical Division, Kyoto University Medical School)
(Chief : Prof. Dr. CHISATO ARAKI)
(Received for Publication : Aug. 4, 1955)

INTRODUCTION

The occurrence of premature puberty in some cases of pineal tumor has stimulated many investigators to study experimentally on the functional relation between the pineal body and the somato-sexual development. The experiments have been carried out mainly by means of either the elimination of the pineal body, or the addition of its effective substance by feeding, implantation, or injection. But the results were conflicting, and the questions have been left open up to date.

Nevertheless it has become an almost universal belief that this organ is an endocrine gland, inhibiting somatic and sexual maturation in some way, and probably interrelated with the hypophysis, which is considered to accelerate the maturation.

In order to reinvestigate these problems, I performed the following experiments.

PART I

FUNCTIONAL RELATION BETWEEN THE PINEAL BODY AND THE HYPOPHYSIS

EXPERIMENT I

i) Procedure : Animals used were male rats and immature at the beginning of this experiment, weighing about 40 g. The first group consisting of 10 rats were castrated and later sacrificed 15, 25, 30, 70 and 100 days after operation respectively. As controls the second group consisting of 10 rats, being almost equal in weight, were kept, without receiving any surgical procedure. They were later sacrificed on the days corresponding to those in the castration group. All pituitaries were weighed, while the pineal body was too small to be weighed, so that the change in weight was roughly estimated macroscopically and microscopically.

Hypophysis, fixed in formalin and embedded in paraffin, was cut at 5 microns into serial sections in the horizontal plane. All sections were stained by ROMEIS' cresazan method.

In each pituitary, the percentages of three sorts of adenohypophyseal cells ······ acidophil, basophil and chromophobe······ were calculated with the modified RASMUSSEN's method (18) ; a section to be counted was chosen from sections near the middle of all section series. Each of sections selected for counting was observed with 1,500 magnifications under oil immersion. The adenohypophyseal cells in every other microscopic field in every other row of fields were counted, as shown by Fig. 1.

In this way about 45 to 65 microscopic fields, a total cell count being about 5,500 to 7,500 cells, were obtained in each hypophysis.

The pineal body was fixed in formalin, embedded in paraffin, cut at 4 microns and stained by haematoxylin-eosin.

ii) Results (Table 1) : The definite increases in weight of the hypophysis and in number and size of the basophilic cells were already found 15 days after castration, and became more remarkable 25 and 30 days postoperatively. Such large basophilic cells were subsequently vacuolated by degrees. These vacuolated basophils, namely "castration cells", were considerably numerous in the hypophyses of rats 70 and 100 days after castration.

Fig. 1 The Calculation Method of the Adenohypophyseal Cells of Rats*

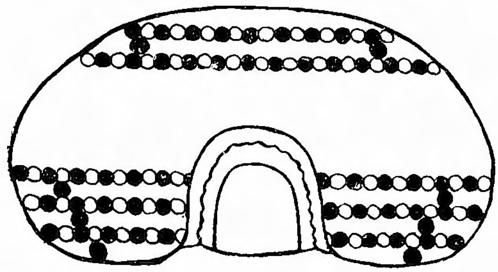


Table. 1. Weight of Hypophysis and Percentages of Three Cell Types in Adenohypophysis of Castrated and Control Rats

Castrated Group							Control Group					
No.	Body Weight (g)	Post-operative Days	Weight of Hypophysis (mg/100g)	Basophil (%)	Acidophil (%)	Chromophobe (%)	No.	Body Weight (g)	Weight of Hypophysis (mg/100g)	Basophil (%)	Acidophil (%)	Chromophobe (%)
1	72	15	5.56	11.0	33.4	55.6	1	64	4.69	7.7	36.7	55.6
2	69	15	5.80	12.8	34.5	56.7	2	71	4.23	10.3	35.8	53.9
3	91	25	6.59	16.3	27.8	55.9	3	95	4.21	8.2	32.4	59.4
4	94	25	6.38	18.0	36.6	45.4	4	93	3.76	8.4	35.6	56.0
5	106	30	6.60	17.4	38.7	43.9	5	101	3.96	9.1	33.9	57.0
6	102	30	5.88	15.2	36.8	48.0	6	98	4.08	7.4	40.2	52.4
7	176	70	5.68	17.6	32.3	50.1	7	177	4.52	8.2	37.7	54.1
8	185	70	5.41	20.3	37.5	42.2	8	171	4.10	7.3	34.6	58.1
9	215	100	5.48	19.4	35.6	45.0	9	206	4.37	9.5	38.1	52.4
10	208	100	5.29	18.6	38.2	43.2	10	208	3.85	8.3	34.3	57.4
$\bar{x}$			5.87	16.7 *	36.5	48.6 *			4.18	8.4	35.9	55.6
s			0.47	2.77	4.14	5.86			0.28	0.9	2.19	2.27
s. e.			0.15	0.88	1.31	1.85			0.09	0.28	0.69	0.72

$\bar{x}$: Mean Value s : Standard Deviation s. e. : Standard Error

* : $P<0.05$, indicating significant difference from control value (Student's t-test).

As Table 1 shows, the abnormal increases in weight of the hypophysis and in percentage of the basophilic cells were observed in all castrated rats. And it can be said from Student's t-test that these increases are significant changes. ($P<0.005$). Moreover if the increase in weight of the hypophysis is taken into consideration, the

* ○ : Microscopic Field to be Counted ● : Microscopic Field not to be Counted

absolute increase of basophilic cells should be more marked. According to the t-test the mean value of the percentages of acidophilic cells in castrated rats did not differ significantly from those of the control group, but as regards the chromophobic cells, the mean value of the percentages in castrated group showed a significant decrease as compared with those of the control group. But if the increase in size of the hypophysis is considered, it is hard to conclude whether the total number of chromophobic cells were really decreased.

These changes of the hypophysis are almost similar to the results of many earlier reporters.

In spite of the definite morphological changes in the hypophysis induced by castration, I was unable to detect any change in the pineal body. Moreover in the size of the pineal bodies roughly estimated macroscopically and microscopically, there were found no plain difference between experimental and control rats.

EXPERIMENT II

i) Procedure : Experimental animals used were 10 immature male rats, weighing about 40 g at the beginning of this experiment, and 4 mature male rats, weighing about 120 g. All these rats underwent pinealectomy, and were sacrificed 21, 30 and 60 days after operation respectively. As controls, 14 immature and mature male rats, almost equal in weight at the beginning of this experiment, were taken, and received no surgical procedure. They were later sacrificed on the days corresponding to those in the pinealectomized group. When they were killed, the hypophyses in all experimental and control rats were studied in the same way as in Experiment I

The method of pinealectomy is as follows :

Ether anaesthesia was used. The skin covering the posterior region of the skull was prepared by shaving and desinfection, and a midline incision was made. Then as shown in Fig. 2, on each side of the sagittal suture, the skull was bored by a

Fig. 2 The Method of Pinealectomy (I)

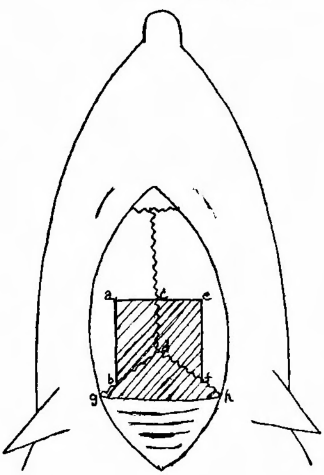


Fig. 3 The Method of Pinealectomy (II)

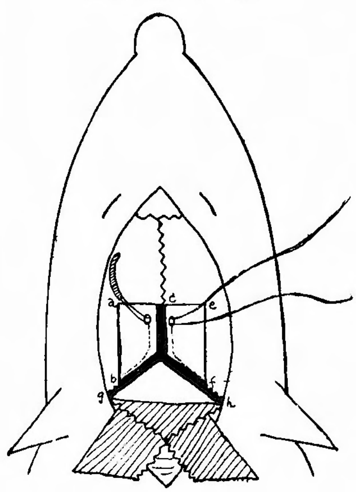
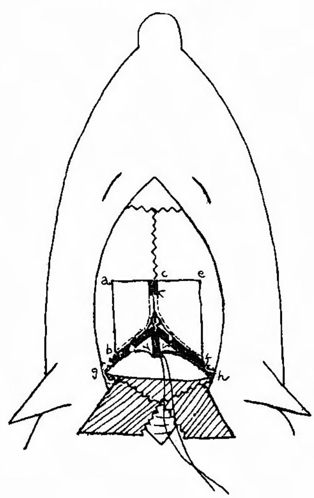


Fig. 4 The Method of Pinealectomy (III)



needle at the points (a) and (e), and incised from each of these two points toward the transverse sutures posteriorly, and toward the sagittal suture medially, the periosteum being scraped only along the incision lines. And then with a small hook two square bone flaps, A (abdc) and B (cdfe), were lifted up and bent posteriorly, when the sagittal suture was separated but the transverse sutures were not, and these bone flaps were attached to the occipital bone with the adherent periosteum. The occipital bone (dgh) was hooked at (d) and raised in one piece posteriorly without being separated from the bone flaps A and B.

The sagittal sinus was now ligated at two sites anterior to the confluence sinuum and divided together with the dura. (Fig. 3). The dura was cut along the perforated lines shown in Fig. 3 and the dural flap continuing to the tentorium was turned down. The pineal body was thus exposed. (Fig. 4). When the tela chorioidea ventriculi tertii was dissected from the cerebrum with an injection needle and the dural flap retracted posteriorly, the pineal body was more or less protruded from its bed near the straight sinus. Then by a small spatula the occipital lobe of the cerebrum was pushed aside, and the stalk of the pineal body was nipped off with a sharp-pointed small pincette. The pineal body was thus totally extirpated. Some haemorrhage always followed the extirpation of the body, but was usually controlled by the light pressure with cotton or gelatin sponge pledgets moistened in

Table 2. Weight of Hypophysis and Percentages of Three Cell Types in Adenohypophysis of Pinealectomized and Control Rats

Pinealectomized Group							Control Group					
No.	Body Weight (g)	Post-operative Days	Weight of Hypophysis (mg/100g)	Basophil (%)	Acidophil (%)	Chromophobe (%)	No.	Body Weight (g)	Weight of Hypophysis (mg/100g)	Basophil (%)	Acidophil (%)	Chromophobe (%)
1	83	21	3.6	11.4	39.2	49.4	1	75	4.0	9.1	35.6	55.3
2	74	21	4.2	9.6	36.3	54.1	2	82	3.7	9.9	41.4	48.7
3	81	21	3.7	8.7	37.4	53.9	3	77	3.9	11.3	38.4	50.3
4	79	21	3.8	7.6	39.8	52.6	4	73	3.4	8.8	35.4	55.8
5	72	21	3.5	10.6	35.7	53.7	5	83	3.6	8.1	41.1	50.8
6	162	21	4.9	9.4	42.3	48.3	6	160	4.4	12.2	32.5	55.3
7	158	21	4.4	9.8	35.3	51.9	7	162	4.3	9.6	36.7	53.7
8	105	30	4.8	9.1	36.5	51.4	8	109	3.7	8.5	42.5	49.0
9	97	30	4.1	8.7	31.5	59.8	9	95	4.2	12.6	36.1	51.3
10	177	30	4.5	9.7	37.5	52.8	10	174	4.6	9.4	37.4	53.2
11	164	60	4.9	10.1	38.9	51.9	11	156	3.9	7.8	40.6	51.6
12	153	60	3.9	9.3	35.6	55.1	12	161	3.7	8.5	34.5	57.0
13	171	60	4.1	8.5	34.3	57.2	13	165	4.9	10.4	32.3	57.3
14	208	60	4.8	7.8	38.1	54.1	14	215	4.7	10.1	41.6	48.3
$\bar{x}$			4.2	9.3	37.0	53.7			4.1	9.7	37.6	52.7
s			0.48	1.01	2.59	2.88			0.44	1.42	3.30	3.00
s. e.			0.13	0.27	0.69	0.77			0.12	0.38	0.88	0.80

$\bar{x}$: Mean Value s : Standard Deviation s. e. : Standard Error hot salt solution. The dural and bony flaps were successively replaced and the skin was sutured.

The extirpated pineal was sandwhiched in the hepar tissue, and immediately put into formalin solution.

ii) Results (Table 2) : In all pinealectomized rats shape and distribution of three kinds of cells in the anterior lobe were unaffected. Also, no significant difference in the quantitative proportion of basophiles, acidophiles and chromophobes was found statistically between pinealectomized and control rats.

Table 2 shows that the weight of the hypophysis or the percentage of the chromophile cells in the anterior lobe in some pinealectomized rats is larger than that in the control rats, while in some others it is smaller. The changes are not significant from the Student's t-test. ($P>0.05$)

EXPERIMENT III

i) Procedure : Twelve rats, weighing about 120 g., were used. Half of them underwent hypophysectomy by Smith's parapharyngeal approach as modified by NAKAO and OMORI, and sacrificed 15, 30 and 60 days after operation. In other six male rats of the control group, the hypophysis was exposed in the same way as above, but left unremoved in place, and the animals were sacrificed at the times corresponding to those in the hypophysectomized group.

The pineal body was always fixed in formalin, embedded in paraffin, and stained with haematoxylin-eosin. In order to compare the changes occurring in the pineal body with those in other endocrine glands, the testes, adrenal and thyroid glands were also weighed and examined histologically.

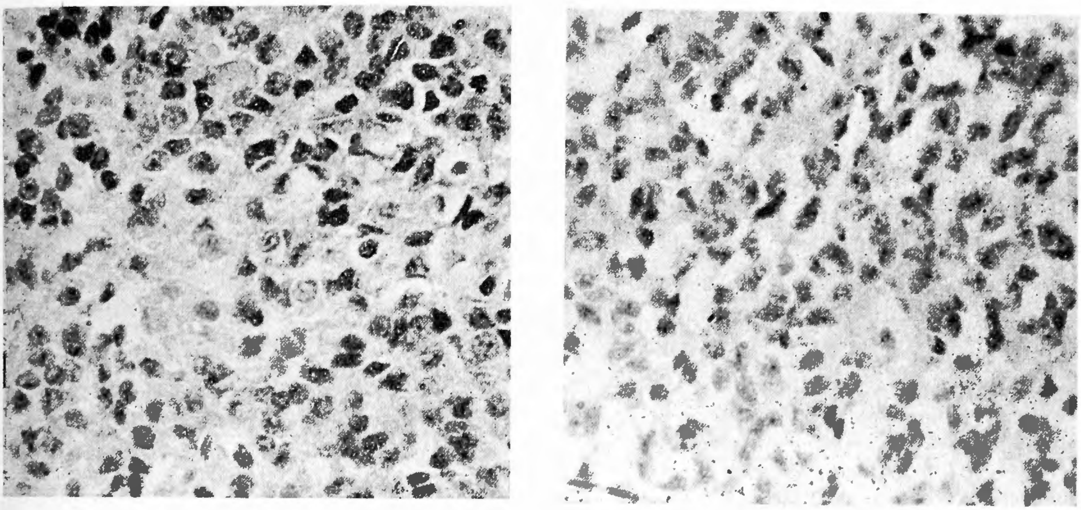
ii) Results (Table 3) : In the hypophysectomized rats 15 days after operation, the testes, adrenal and thyroid glands were found to be smaller in weight than those in controls. (Table 3)

Table 3. Weights of Suprarenals, Thyroids and Testes in Hypophysectomized and Control Rats

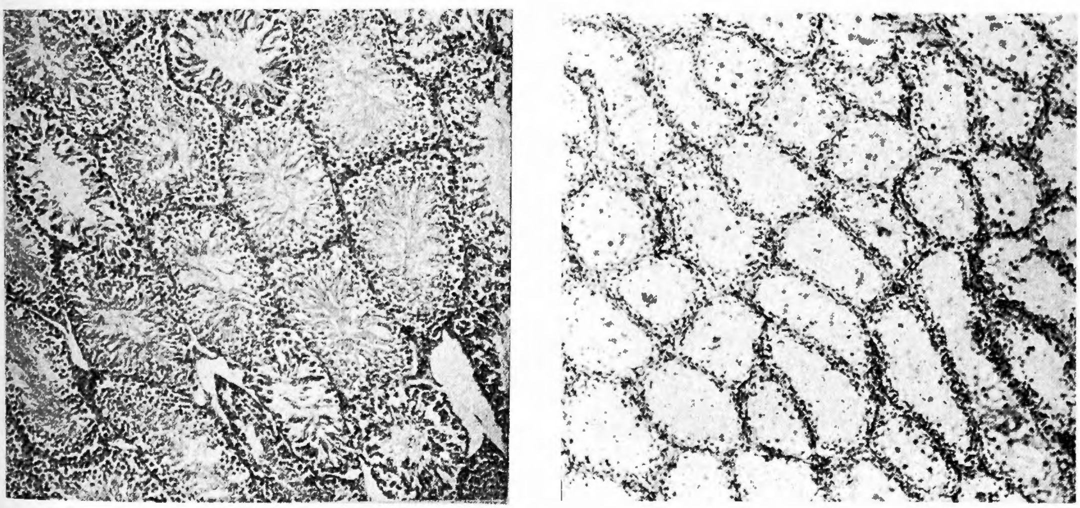
	No.	At Operation: Body weight (g)	At Operation: Postoperative Days	At Necropsy: Body Weight (g)	Weight of Both: Suprarenals (g)	Weight of Both: Thyroids (g)	Weight of Both: Testes (g)
Hypophysectomized Series	1	127	15	123	0.014	0.012	0.735
	2	135	15	129	0.013	0.011	0.665
	3	120	30	106	0.009	0.008	0.435
	4	123	30	110	0.008	0.008	0.272
	5	129	60	94	0.007	0.008	0.245
	6	115	60	96	0.008	0.010	0.284
Control Series	1	125		158	0.034	0.019	1.985
	2	130		160	0.038	0.021	2.036
	3	118		175	0.040	0.023	2.100
	4	125		184	0.044	0.020	2.262
	5	127		213	0.042	0.032	2.434
	6	120		197	0.043	0.035	2.213

Fig. 5 The Pineal Body, Testis, Adrenal and Thyroid Gland in the Hypophysectomized and Control Rats.

Control Rat No. 5 Hypophysectomized Rat No. 5



(Pineal Body)

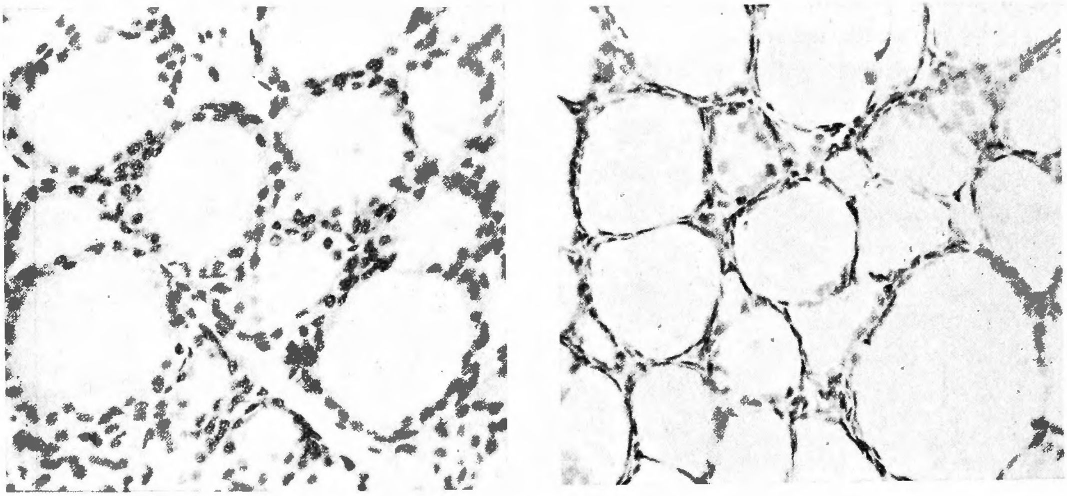


(Testis)

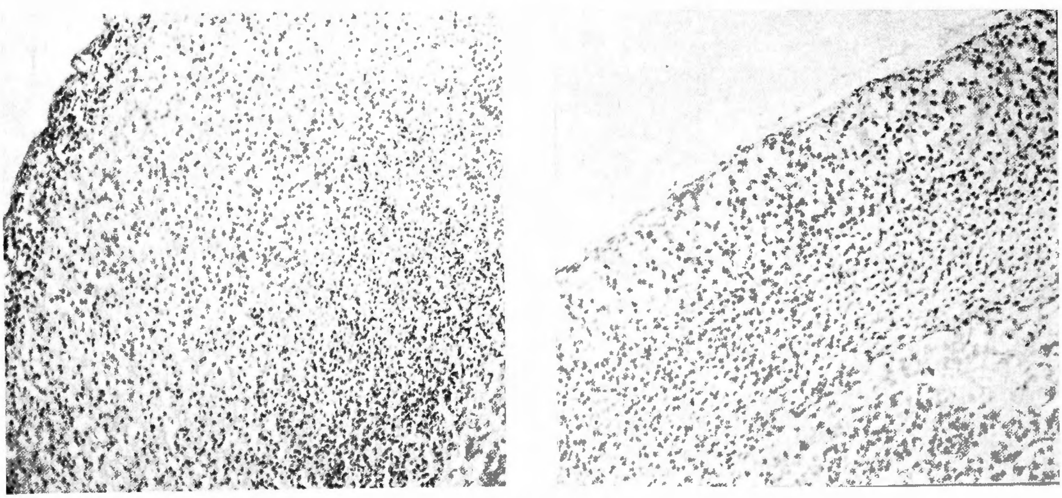
Microscopic examinations revealed that in testis the germepithelium was very atrophic with no spermatogenesis, and in adrenal the cortex, especially zona fasciculata, was remarkably atrophic, and that in thyroid the acini were of smaller size and lined by flattened epithelium. (Fig. 5).

The longer the period after operation was, the more remarkable these changes were. Therefore in the hypophysectomized rats sacrificed 30 or 60 days after operation, testes were too small to be palpable transcutaneously.

On the other hand, the pineal body surely became smaller in size than the normal one, but in slighter degree in comparison with the other endocrine organs ; that is, after 15 and 30 days following hypophysectomy, the volume diminution of



(Thyroid Gland)



(Adrenal Gland)

the pineal was not yet so marked, and as late as after 60 days it was considerable. From the microscopic examinations of such small pineal bodies, it became clear that this organ was fairly atrophic ; the parenchymal cells decreased in number and size, though they suffered no change in form and distribution, and the interstitial cells relatively increased in number.

DISCUSSION

In the first place, the considerable morphologic changes could be recognized in the hypophysis after castration, but not in the pineal body. This fact proves that the pineal body does not show any morphological change not only against the elimination of the testicle itself, but also against the successive changes of the hypophysis following castration.

There have been several investigators who studied the histologic changes of the pineal body after castration, but the findings reported have been diverse : some found the atrophy of the parenchymal cells and hardening of the interstitial tissue (2, 6), others observed contrarily the hypertrophy of the parenchyma (17), while still others recognized no change (12). In my experiment, I could recognize no change in spite of my every possible effort.

Of course it may be unreasonable to expect that every change in the function of the pineal body might be caught morphologically, especially by haematoxylin-eosin stain, but at least it can be asserted that the positive morphological changes (by means of haematoxylin-eosin stain) in the pineal body reported by earlier investigators were not confirmed by the present study.

In the second place, comparing with the considerable changes in the hypophysis of the castrated rats, I have recognized no change in it after the ablation of the pineal body, which has been supposed to have an interrelation with the hypophysis as the testis has. Regarding the histologic changes in the hypophysis after pinealectomy, some investigators found the increase of eosinophilic cells (11), and others recognized the hyperplasia of basophilic cells (17). But none of them did exactly count the percentages of the three sorts of cells in the anterior pituitary.

It is often experienced that the roughly estimated increase or decrease of these three sorts of cells is fairly different from the exact ratio counted quantitatively. To keep off this mistake, I applied RASMUSSEN's method in the human hypophysis to the rat's hypophysis. However between pinealectomized and control rats, any significant difference could not be recognized.

In the third place, I recognized that hypophysectomy caused the specific atrophy of parenchymal cells in each of peripheral endocrine glands in addition to the remarkable reduction of the weight of these organs. Such changes occurred very soon, already only a half of month after hypophysectomy. On the other hand, the volume diminution of the pineal body after hypophysectomy was not so marked as that of other endocrine organs, and appeared more slowly. Histologically, the parenchymal cells certainly decreased in number and size, but I could recognize no change in form and distribution of them.

After hypophysectomy rats nearly stopped their growing, and various organs besides the endocrine, such as pancreas, liver, spleen and etc., decreased their weight and became considerably atrophic. Brain was atrophic, too. Therefore it is impossible to decide whether the volume diminution of the pineal body results from the specific effect or overall effect of hypophysectomy. But as compared with the thyroid, adrenal and testis which had been considered to have close connection with the hypophysis, the pineal body needed a longer time to diminish its volume and the change of parenchymal cells was less marked. Considering also that I could recognize no change in the hypophysis of pinealectomized rats, I assume that the change of the pineal body after hypophysectomy might be general changes.

PART II

PRECOCIOUS PUBERTY AND PINEAL BODY

The problem, whether or not the pineal body releases a hormone which inhibits the somatic and sexual development, has been studied by means of (i) elimination of the body, or (ii) addition of its extracts. In the latter method, however, it is very difficult to determine whether or not the changes brought about are surely due to the effect of a hormone from that organ, since so many factors have to be considered, i. e. the process of making the extract, the dosage and duration of administration, and the way how to choose the controls etc. And the administration of extracts to normal animals causes only a relative increase of the effective substance, whereas the extirpation of the organ means the absolute disappearance of it. Therefore the latter method seems to be preferable, although somewhat difficult technically.

By the way Lowenstein (14), reviewing the earlier experimental results about the pineal body, stated that there had been 13 positive and 12 negative results after pinealectomy, and 11 positive and 22 negative results after addition of the pineal substances. More frequent occurrence of negative results in the latter experiments may probably be due to artificial factors in experimental procedures. For these reasons, I have done pinealectomy in this experiment.

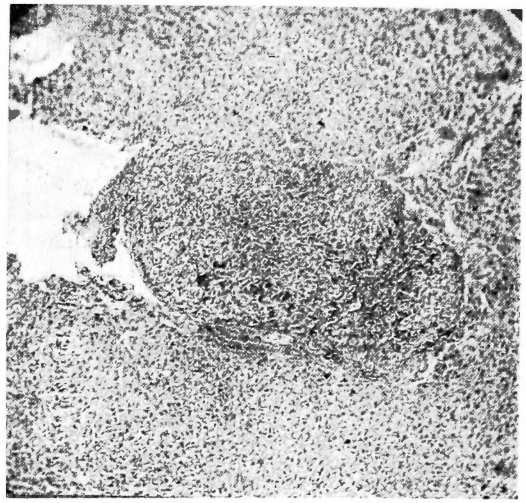
i) Procedure : The male rats, when operated on, varied from 5 to 7 weeks in age, weighing from 35 to 60 g. and all animals of pinealectomized and control groups were members of the same litter. 10 litters, 36 rats, were used. 20 rats of them were pinealectomized and remaining 16 were used as controls. There were two types of controls, the one consisting of 5 rats (I … III groups) being the non-operated control and the other consisting of 11 rats (IV … X groups) being the operated control. The pinealectomy was performed following the technique as described in Part I, Experiment 2. For the animals of the operated control, the pineal body was exposed in the same way as in pinealectomy but left unremoved in place.

The I … VI groups were killed 6 weeks and the VII … X groups 13 weeks after operation. All experimental and control rats were kept and fed under identical conditions, the cage of each rat being of the same size and in the same situation, and the amount of food being equal. These animals were weighed weekly, and at the time of autopsy, testes were weighed and histologically examined by haematoxylin-eosin stain. Each extirpated pineal was examined histologically, after sandwiched in the liver tissue and it was confirmed whether or not the removal of it was complete. Also the rest brains of all pinealectomized rats were fixed in formalin, and the pineal region was serially sectioned to search for remnants of pineal tissue and for damages to neighboring structures by the operation.

ii) Results : Four rats, of which two underwent pinealectomy and the other two control operation, died of pneumonia or brain abscess before the end of the experiment, and were excluded from the data. Thus, when sacrificed, experimental rats were 32, consisting of 18 pinealectomized, 5 non-operated and 9 control-operated.

Extirpation of the pineal body in each rat proved to have been accomplished quite satisfactorily. Namely, as shown in Fig. 6, all extirpated pineal bodies were entirely covered with a capsule of connective tissue, and besides, serial sections cut through the pineal region revealed no trace of remaining pineal tissue.

Fig. 6 Extirpated Pineal Body Sandwiched in Liver Tissue



Moreover there were found no gross lesions in the diencephalon and midbrain, which might have some relation with precocious puberty. Only occipital lobes of the cerebrum in each case were more or less destroyed. However such destruction in the occipital lobes were nearly lacking in several cases where the operation was done quite successfully. In all cases the stalk of the pineal was completely cut off from the habenular commissure. In the sections caudal to this point the pineal tissue was entirely lost. The meninges left in this region, were, to some extent, thickened and infiltrated by leucocytes and there were phagocytes, including haemosiderine, in the meninges or in the choroidal plexus as a trace of the past inflammatory changes. These canges were most remarkable in the region where the pineal stalk was considered to have been cut off. And vena Galeni, tightly adhering to the pineal body, cannot be separated from it without injury. Thus, this vein was always torn off at pinealectomy. But the bleeding was easily controlled. Except those, no change was found in other parts of brain, i. e. habenular and posterior commissures, habenular nucleus, thalamus, hypothalamus and midbrain.

As shown in Table 4, for the first 3 weeks after operation average increase of the body weight in the pinealectomized rats was slightly less than that in the control rats, but contrarily, for the second three weeks (from the 4th to 6th week), the former became a little more than the latter. Nevertheless, if Student's t-test is tried, these differences are not significant. ($P > 0.05$). And for the next 7 weeks (from the 7th to 13th week), there were no recognizable differences between the two.

Besides, no significant differences were seen in the testicular weights between the two groups (Student's t-test), and also no histologic change of the testis common to the pinealectomized series was observed.

DISCUSSION

From my experiment it was evidenced that, after the pineal body was totally extirpated with the slightest injury to the neighborhood, there was recognized no significant change in the growth of the body and in the weight of the testes. Then, why do these results differ so greatly from the reports of some others in

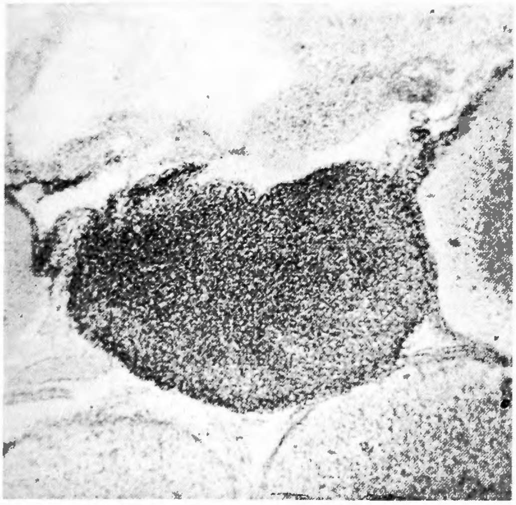
Table 4. Body Weight and Testicular Weight of Pinealectomized and Control Rats.

		No.	Body Weight (g)							Testicular* Weight (mg/100g)
			On Operation (A)	After Operation 3 Weeks (B)	6 Weeks (C)	13 Weeks (D)	(B)—(A)	(C)—(B)	(D)—(C)	
Pinealectomized Group	Group I	1	52	98	143		46	45		1.17
		2	50	96	132		46	36		1.19
	Group II	3	62	116	153		54	37		1.17
		4	57	105	141		48	36		1.16
	Group III	5	51	106	149		55	43		1.18
	Group IV	6	46	103	138		57	35		1.27
	Group V	7	32	67	111		35	44		1.15
		8	35	73	118		38	45		1.19
	Group VI	9	42	83	126		41	43		1.18
	Group VII	10	44	86	128	190	42	42	62	1.30
		11	46	87	135	198	41	48	63	1.26
	Group VIII	12	42	89	128	190	47	39	62	1.29
		13	40	88	124	182	48	36	58	1.18
		14	39	86	125	186	50	34	61	1.18
	Group IX	15	49	102	132	194	53	32	60	1.24
		16	44	99	131	189	55	32	58	1.28
	Group X	17	35	85	125	189	50	40	64	1.28
		18	33	82	118	179	49	36	61	1.27
	$\bar{x}$						47.6	39.1	61.0	1.22
	s						6.1	4.7	1.9	0.051
	s. e.						1.4	1.1	0.7	0.012
Control Group	Group I	1	50	95	129		45	34		1.11
		2	51	95	130		44	35		1.09
	Group II	3	62	108	146		46	38		1.18
		4	64	112	148		48	36		1.17
	Group III	5	50	102	145		52	46		1.18
	Group IV	6	51	103	135		52	32		1.19
	Group V	7	32	72	112		40	40		1.17
	Group VI	8	42	85	132		43	47		1.23
	Group VII	9	44	90	128	198	54	38	70	1.29
	Group VIII	10	43	89	131	188	56	42	57	1.22
		11	41	92	125	183	51	33	58	1.21
	Group IX	12	47	101	136	196	54	35	60	1.30
	Group X	13	36	87	123	182	51	36	59	1.23
		14	32	83	120	187	51	37	67	1.21
	$\bar{x}$						49.1	37.8	61.8	1.20
	s						4.1	4.4	4.9	0.056
	s. e.						1.1	1.2	1.3	0.015

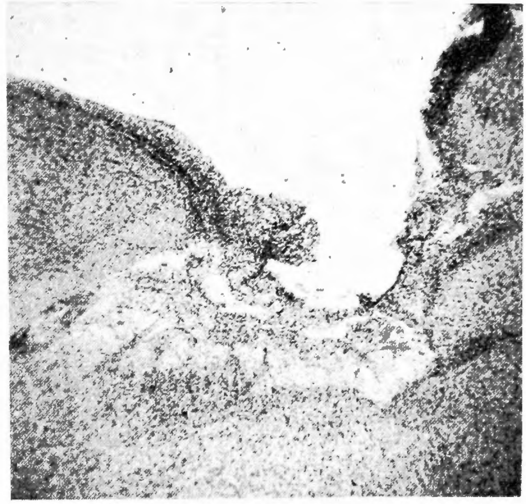
* … Weight of both testicles

$\bar{x}$ … Mean s … Standard deviation s.e. … Standard error

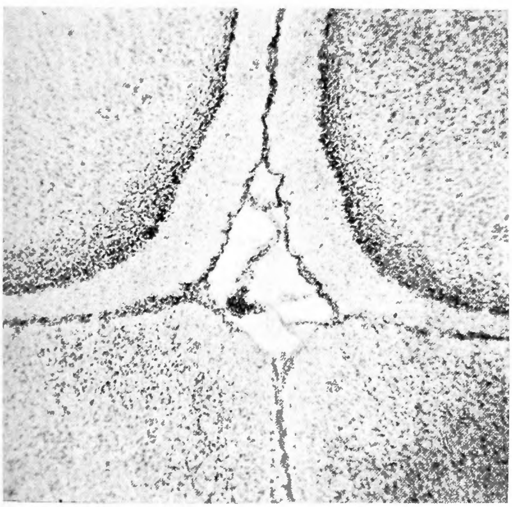
Fig. 7 Serial Sections of the Pineal Region of the Brain in Pinealectomized and Control Rats.



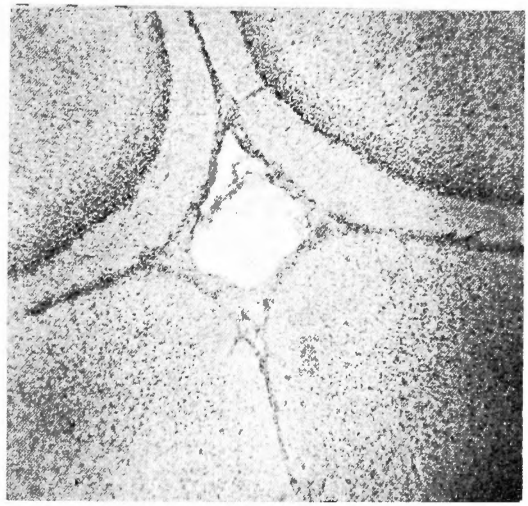
(1)



(1)'

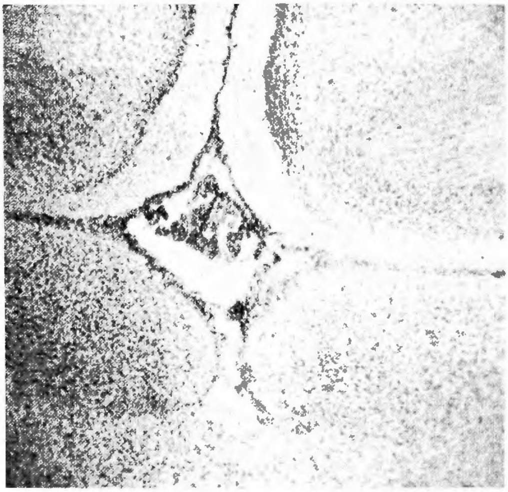


(2)



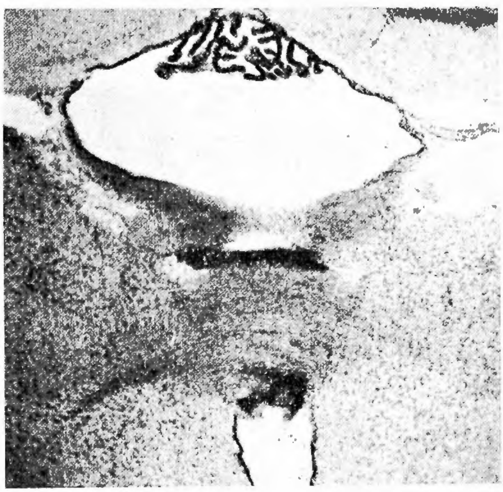
(2)'

(1), (2), (3), (4) and (5) ··· Pineal region of the control rat (Group VIII, No. 11)
(1)', (2)', (3)', (4)' and (5)' ··· Pineal region of the pinealectomized rat (Group VIII, No. 13)
(1), (1)' ··· Caudal part of the colliculus rostalis of corpora quadrigemina. Plate (1)' shows some round cell infiltration in the considerably thickened meninges in the complete absence of the pineal body.
(2), (2)' ··· Middle part of the colliculus rostalis. In (2) the cranial pole of the pineal body is definitely present, while in (2)' one can only see the thickening of the surrounding meninges.
(3), (3)' ··· Cranial part of the colliculus rostalis and posterior pole of the third ventricle. In this region the most remarkable changes are observed, i.e. as shown in plate (3)', considerable adhaesion of the meninges and cell infiltration are present, suggesting that the pineal body should have been cut off here.
(4), (4)' ··· Commisura habenularis and commisura posterior are seen. Between these two plates, there is no definite difference except the presence of few cells containing haemosiderine in the choroidal plexus of (4)'.
(5), (5)' ··· In these plates nucleus habenularis is seen, and no definite difference is observable between the two.



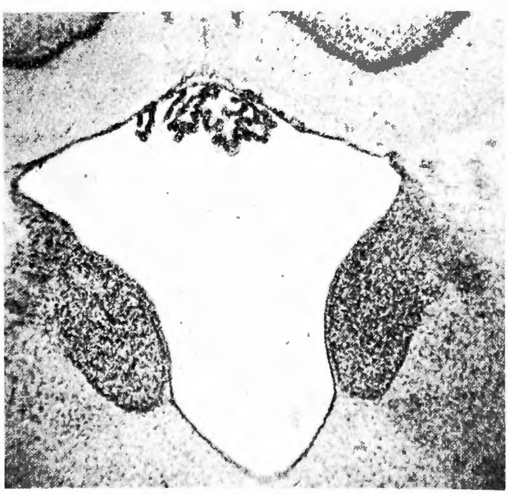
(3)

(3)′

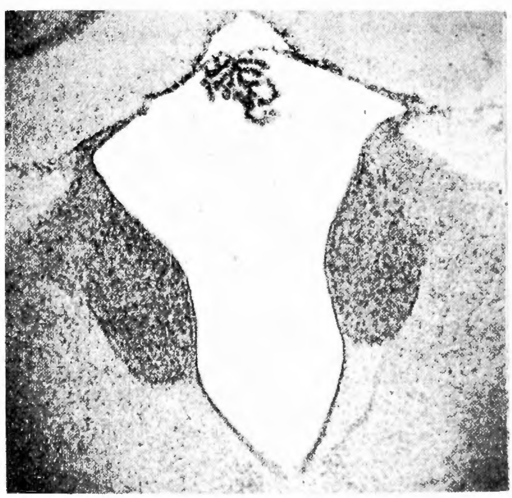


(4)

(4)′



(5)



(5)′

which the definite changes had been recognized ? In order to answer this question so many factors should be taken into consideration, such as the experimental control, the selection of the experimental animals, the operative mortality, the age at operation, the completeness of the extirpation, the extent of damages to the neighboring nervous structure and the way of the interpretation of the data.

i) Experimental control : In some of the previous reports there are some questions as to the selection of the control, because control animals are very few in number, or they are not of the same litter as the pinealectomized.

As the cotrol, I used as many as possible of the animals of the same sex and the same litter as the pinealectomized group.

ii) Selection of experimental animals : I used rats as experimental animals because of the following advantages. The litters are numerous and their growth curves are relatively stable. And as compared with the cerebrum of a dog, cat and rabbit, that of a rat is poorly developed, whereas the corpora quadrigemina are highly developed, so that the pineal body is situated more superficially, thus the extirpation being easier. In guinea-pigs, in which the litters are few, it is hard to get a large series of the experimental and control animals. There have been some reporters who used chickens as experimental animals, but because of the wide variation in the growth curve of them, I do not think they are preferable to rats.

iii) Operative survival : Operative mortality of pinealectomy has been so high that it is sometimes difficult to obtain a large series of pinealectomized animals sufficient to allow a critical analysis of the data. It is more than 50 per cent in several previous reports (1, 9). The chief causes of death were haemorrhage, infection and crush of the neighboring nervous tissue. These causes could be avoided by practice and carefulness, and consequently I have been able to accomplish the complete extirpation of the pineal body with the minimum operative mortality, only about 1 per cent.

iv) Age at operation : I used for pinealectomy young male rats soon after the weaning period, weighing about from 30 to 60 grams. There is no doubt about that the younger the rat is at operation, the more marked the difference in the growth curve may come out. For this reason ANDERSEN (1) and WAKITA (20) extirpated the pineal body of the rat 1 … 3 days after birth in their studies. But I think it is not desirable to operate before the weaning. Because, at first the operative mortality would be very high … it was the case in the experiments of both ANDERSEN and WAKITA … and in the second place it is impossible to keep the rat in different cage one by one. They must be bred together with their mother and litter mates, and then the amount of food taken will be different in each rat, and they are apt to suffer from bites and scratches of the operation wound by the other animals, especially by their mother, and thus their growth might be influenced on heavily.

In order to avoid such disadvantages, I used the rat soon after the weaning period. I don't think this stage is too late, judging from the results of many earlier experiments.

v) Completeness of the extirpation : It is generally accepted that even if only a little part of the endocrine organ is left, no fall-off sign does appear because of the compensation of the function. Therefore in case of pinealectomy, it is very important to investigate whether the ablation of the organ is complete or not. For this reason I examined carefully each extirpated pineal body and each brain of the pinealectomized rats histologically. A few earlier reporters investigated the completeness of the ablation histologically (7, 9), but many others did not.

vi) Extent of damages to the neighboring nervous structures : It has been said that precocious puberty tends to occur in such cases of pineal tumors as have extended to the hypothalamus, or as have led to an obstructive hydrocephalus, resulting, of neccesity, in secondary hypophyseal disturbances. And BING, GLOBUS and SIMON (20) pointed out in an analysis of collected cases of precocious puberty associated with pineal tumors that in 70 per cent there were symptoms or signs referable to hypothalamic involvement.

Moreover precocious puberty has been often noticed in the patient who had a third ventricle tumor and whose pineal body was intact (3).

HANDA (8) and KUROZAWA (13) of our laboratory pointed out the facts that in both the male chickens and the female rats, whose pineal bodies had been damaged, the somato-sexual development was nearly normal, whereas in the chickens and rats in which the neighboring nervous structure had been damaged, the somato-sexual development was retarded sometimes and accelerated other times.

Judging from the facts mentioned above, I think that in case of pinealectomy the extent of damage to the neighboring structures should be investigated carefully. Although there were some who had described about the perfection of extirpation, I could not find any report in which the extent of the destruction of the neighboring structures had been shown. I think that those who drew the positive conclusion should describe the extent of damage to the neighboring structures, and on the contrary those who gained the negative should prove the completeness of extirpation.

Since in my study the microscopic examination of the pineal region has revealed no structural change in the thalamus, hypothalamus and brain stem after pinealectomy, I think that these parts could not have any special effect on the somato-sexual development in my animals.

Another fact which must be considered here, is that the straight sinus or vena Galeni has always been torn off at pinealectomy, but not at control operation. For a fairly long time, it has been assumed that a hydrocephalus results from the obstruction of vena Galeni, and that the hydrocephalus changes the function of the hypothalamus or the hypophysis in the direction usually hypopituitarism and towards rarely towards pubertas praecox.

Bedford proved from his experiment in dogs and monkeys that no hydrocephalus was caused by the ligation of vena Galeni, owing to collateral circulation. In my study, there was found no trace of internal hydrocephalus … dilatation of the ventricles … in the brains of all pinealectomized rats. Then, I think that in rats no internal hydrocephalus comes about after the ligation of vena Galeni and that somato-sexual development is not influenced on by this ligation.

In my experiment, after pinealectomy there was not found any considerable side-damage in the brain, except the above mentioned injuries of vessels, meninges and occipital lobe, which may be considered to be minimal lesions at pinealectomy.

Since I opened the skull as a bone flap and put it back in its place, and since postoperatively the experimental animals were segregated one by one, injury from outside could be avoided almost surely, and good bony conjunction was established, and fur grew again. Consequently, 3 weeks after operation, it could no more be distinguished at a glance, whether the rat had undergone the operaion or not. Some of the past reporters did not replace the skull after operation.

vii) The interpretation of the data : There have been some earlier investigators who might have made a mistake in interpreting the results. ANDERSEN and WOLFE (1) pointed out that the positive results which had been reported by several investigators, viz. IZAWA, FOÀ, HORRAX and etc., should be negative from the statistical calculation or the critical analysis of their data.

In my animals, after pinealectomy some grew more quickly and some others more slowly than controls, and the remaining grew equally to controls. And the testicular weight was larger in some of the pinealectomized animals than in the control, but smaller in some others. Thus I have decided the results to be negative. In this connection some comments will be made in the following.

In the first place it must be emphasized that the complete extirpation of the pineal body was proved microscopically in my cases. Therefore it should be expected that the common cause, viz. the ablation of the pineal body, has to bring about the common symptoms in all experimental animals. And some changes which are found only in some cases, should not be attributed to the common cause.

In the second place, I used the Student's t-test for the evaluation of my data, and I could not find any significant difference in both the body weight and the testicular weight between the two groups. Of course at the beginning of the experiment the body weight of rats differed so much from each other that t-test is not easily applicable for the comparison of changes in the body weight. But in the growth curve of rats from 30 to 180 g. the increase rate of the body weight became generally regular in the elapse of time, and so I think the Student's t-test may be available for the estimation of these changes to some extent.

In the third place, the data cannot be regarded as significant, also from SNEDECOR's Table about binomial distribution.

Of course I don't think that the comparison of the two growth curves can always be done satisfactorily by the statistical calculation, but I believe the interpretation of the data based on the statistical calculation as I have described is the most reasonable and therefore should be tried.

The assumption that a certain organ is an endocrine organ must be based on convincing evidences showing that the organ secretes a hormone. If such evidences are not given, the organ should not be included in the category of endocrine organs. For instance, it is for such a reason that the heart and liver are not regarded as endocrine organs. For the same reason, the pineal body is not an endocrine organ, so far as the results of earlier experiments were rather negative. From the results of my elaborate experiments I have come to the following conclusion : it is unreasonable to assume that the pineal body has some suppressive function against the somato-sexual development probably in the antagonistic relation with the hypophysis.

SUMMARY

The results of my experiments in immature male rats can be summarized as follows.

1) Two weeks after castration, both the weight of the hypophysis and the number and size of basophilic cells in it were found to be increased. Then on the 70th and 100th day after castration vacuoles came about in the hypertrophied basophils.

Thus a series of typical changes occurred in the hypophysis, while on the contrary in the pineal body any significant histological change was not found at all.

2) After pinealectomy, there was found no change in the weight of the hypophysis, nor in the shape and percentages of the adenohypophyseal cells.

3) After hypophysectomy, the thyroid, adrenal and testis become atrophied remarkably with intense degeneration of parenchymal cells. Also the pineal body undergoes some atrophy, that is, the volume is reduced by degrees and also parenchymal cells decrease in number and size. But in considering that the changes are not so distinct as in other endocrine organs, any special relation between the pineal body and the hypophysis can not be established.

4) No difference between the pinealectomized and the control rats was recognized in the body weight and the testicular weight, and in the histological finding of the testicles too.

In my experiments, I have devised my own operative technique for pinealectomy and given attention to the completeness of the removal and to the minimal extent of the destruction of the neighboring nervous structures.

References

1) Andersen, D. H. and Wolfe, A. : Pinealectomy in Rats, with a Critical Survey of the Literature. J. Physiol., **81** : 49, 1934. 2) Aschner, B. : Schwangerschaftveränderungen der Zirbeldrüse. Zbl. f. Gyn., **37** : 840, 1913. 3) Bauer, H. G. : Endocrine and Other Clinical Manifestations of Hypothalamic Disease. J. Clin. Endocrinol., **14** : 13, 1954. 4) Bedford, T. H. B. : The Great Vein of Galen and the Syndrome of Increased Intracranial Pressure. Brain., **57** : 1, 1934. 5) Bedford, T. H. B. : The Venous System of the Velum Interpositum of the Rhesus Monkey and the Effect of Experimental Occulusion of the Great Vein of Galen. Brain., **57**, : 255, 1934. 6) Biach, P. und Hulles, E. : Über die Beziehungen der Zirbeldrüse zum Genitale. Wien. Klin. Wschr., **25** : 373, 1912. 7) Davis, L. and Martin, J. : Results of Experimental Removal of Pineal Gland in Young Mammals. Arch. Neurol. & Psych., **43** : 23, 1940. 8) Handa, H. : Experimental Studies on the Function of the Pineal Region Controlling the Somato-sexual Development in Male Chickens. Acta Scholae Med. Univ. in Kioto, Japonica, **31** : 143, 1953. 9) Horrax, G. : Studies on the Pineal Gland. I. Experiment-

al Observations. Arch. Int. Med., **17** : 607, 1916. 10) Hortega, D. : Pineal Gland, Penfield's Cytology and Cellular Pathology of the Nervous System, **2** : 235, 1932. 11) Ikuta, H. : Über Experimentelle Studien der Zirbeldrüse. (II Mitteilung) Morphologische Studien über die Hypophyse bei Pinealektomie. Trans. Soc. Path. Jap., **28** : 420, 1938. 12) Kolmer, W. und Löwy, R. : Beiträge zur Physiologie der Zirbeldrüse. Arch. f. Ges. Physiol., **196** : 1, 1922. 13) Kurozawa, M. : The Function of the Pineal Body and Its Neighboring Structures for the Control of the Somato-sexual Function. Experimental Studies in Female Rats. Folia Psych. et Neurol. Jap. (To be Published.) 14) Lowenstein, M.G. : Studies on the Function of the Pineal Body. Exp. Med. & Surg., **10** : 135, 1952. 15) Maeda, M. : Über Einwirkung von Pineal (Präparat aus der Zirbeldrüse des Rindes), Oophormin (Präparat aus dem das Corpus Luteum Entfernten Rindsovarium) und Thyroid (Präparat aus der Schilddrüse des Schäfchens) auf die Zellen des Hypophysenvorderlappens beim Kaninchen. Okayama-Igakkai-Zasshi, **44** ; 2837, 1932. 16) Moszkowska, A. : Contribution a L'etude de L'antagonisme Epiphysohypophysaire., J. Physiol., **43** : 827, 1951. 17) Nakashita, S. : Studies on the Pathogenesis of the Deficiency Syndromes Following Castration., Folia Endocrinol. Jap., **26** : 275, 1951. 18) Nakashita, S. : Studies on the Precocious Mechanism of the Male Genitals Caused by the Pinealectomy., Folia Endocrinol. Japonica., **27** : 72, 1951. 19) Rasmussen, A. T. : The Percentage of the Different Age of Cells in the Male Adult Human Hypophysis., Amer. J. Path., **5** : 263, 1929. 20) Wakita, K. : Experimental and Clinical Study on the Pineal Body., Mitteil. Jap. Ges. f. Gyn., **38** : 835, 1943. 21) Weinberger, L. M. and Grant, F. C. : Praecocious Puberty and Tumors of the Hypothalamus., Arch. Int. Med., **67**: 762, 1941. 22) Wislocki, G. B. and Dempsey, E. W. : The Chemical Histology and Cytology of the Pineal Body and Neurohypophysis., Endocrinol., **42** : 56, 1948.

和文抄録

松果体と脳下垂体との関係－特に身体並びに生殖器の発育に就て

景　山　直　樹

(京都大学医学部外科学第１講座　主任：荒木千里教授)

松果体が身体並びに生殖器の発育を抑制するホルモンを出すと云う説が広く信じられ，更には之を前提として，松果体が，機能的に反対と考えられる脳下垂体と関係があるらしいと云う臆測が生れた．之等に関する実験は従来数多行われているが，その結果は必ずしも一致していない．

私はその間の消息を検討する目的から，幼若或いは成熟雄白鼠を用いて実験を行い，次の如き結果を得た．

第１に去勢後100日間の観察の結果，下垂体は術後２週頃より重量が増し，且つ Basophil 細胞増殖が現れて来る．更に70日頃からその肥大せる Basophil 細胞に空胞化が起る．この様に下垂体は一連の定型的な変化を示すが，之に対する松果体は組織学的に何等変化を認め得なかつた．

第２に松果体摘出後２ヶ月間の観察では，下垂体の重量，下垂体各種細胞の大きさ，形状並びに比率（Rasmussen法を応用して計算す）に何等変化を認めなかつた．

第３に下垂体剔出後２ヶ月間の観察では，末梢性内分泌器官である甲状腺，副腎，睾丸等は著しく萎縮し，且つ著明な変性変化を認める．松果体も矢張り漸次その容積を減少し，組織学的にも実質細胞の減少，縮小等を認めるが，之等の変化は上記３器官の変化に較べると可成り軽度であり，従つてその変化が下垂体剔出による直接的影響の為か，同操作に依つて起る全身性変化の部分現象なのか，決定し得なかつた．

第４に10群の同腹白鼠の雄のみ選び，その半数の松果体を剔出し，残りの半数を対照（手術を行わないもの，試験開頭のみのもの等）とし，術後６週乃至13週間の観察の結果，体重，睾丸重量共対照群との間に有意な差を認め得ず，又睾丸の組織学的所見も対照と差異を認めなかつた．尚その際剔出せる松果体，剔出後の脳（松果体部）を組織学的に検索したが，全例，松果体は完全に取れて居り，且つ周囲神経組織に対する障碍は僅少で，問題となる視床下部，視床，脳幹部等には殆ど器質的障碍を認めなかつた．

以上の結果から松果体が身体並びに生殖器発育抑制機能があると云う事も，又下垂体と特殊な連関を持つと云う事も，極めて疑わしいと結論する．